A Research proposal

"NANOTECHNOLOGY BASED PLATFORM FOR DETECTION OF VITAMIN D"

Submitted to

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Swami Ramanand Teerth Marathawada University, Nanded for

the consideration of Registration for the Degree of Doctor of Philosophy

In the Faculty of Pharmaceutical Sciences and Technology

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2022-2023

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ABSTRACT

Vitamins are micronutrients and has required for maintain the healthy metabolic function. vitamins play important role in various physiological functioning, biological responses, and host immunity. Thus deficiency of vitamins (Hypovitaminosis) leads to developed various conditions like inflammatory disease, allergy, infection, rickets, poor growth etc. while Toxicity (Hypervitaminosis) leads to diarrhoea, calcification of soft tissue, haemolytic anaemia, Double vision etc. vitamins needed by the body in very small amounts. However, their impact on a body's health are critical, and deficiency in any of them can cause severe and even life-threatening conditions. Thus rapid & accurate quantification of level of vitamin has attracted much attention. Compared with traditional analytical methods such as Atomic absorption spectroscopy, UV-Visible spectroscopy, Colorimetric analysis, Atomic Emission Spectroscopy photoimmuloscence, chemimmuloscence etc. these techniques are complex, time consuming and expensive therefore there is need to developed new method such as biosensor for detection of vitamins. Biosensors are rapid, simple & inexpensive device with high sensitivity and bio recognition element for analyte specificity. So using this technique detection of vitamin D can be made at ease and rapid.

Keywords: Micronutrient, Vitamin D, Nanotechnology, Biosensor, Biorecognition

1. INTRODUCTION

1.1 INTRODUCTION TO VITAMIN

Vitamins (Vita-Life, amines- organic compound) are compound that are essential in very small amount to maintain normal Functioning and healthy body metabolism of Carbohydrate, fat. They are not synthesised by body & must be provided through dietary intake. As vitamins required in micro quantity they are said micronutrients. vitamins are 1st discovered by Hopkins in 1907, & named by Funk in 1911. Later McCollum & Davis showed that some vitamins are fat soluble (A, D, E, K) & water soluble (B, C). water soluble vitamins are not stored in body & get readily excreted as they are water soluble, while fat soluble vitamins are stored in liver for reserved & used latter when they need. Most water soluble vitamins needed for formation of coenzymes, which are needed by some enzymes for catalytic activity (TCA or Krebs cycle). Inadequate intake of vitamins (Hypovitaminosis) leads to serious health issues (Anaemia, Beriberi, pellagra etc.) & excessive vitamin (Hypervitaminosis) may lead to be harmful effect (mouth ulcer, & confusion, Diarrhoea, Weight loss, Calcification of soft tissues etc.) (1).

Interestingly, in case of vitamin D, by sufficient exposure to ultraviolet (UV) light, adequate levels can be derived through synthesis within the skin. Therefore, inadequate diet and lack of exposure to sunlight could result in the development of vitamin D deficiency (2).

One of the key hormones for maintaining overall health and preventing chronic diseases is vitamin D, yet it is believed that one billion individuals globally have vitamin D deficient or insufficient. Additionally, to having a major impact on bone and muscular health, numerous studies have shown that it can lower the chance of developing common malignancies, diabetes, autoimmune, infectious, and cardiovascular illnesses.

Since vitamin D is only naturally present in a few number of meals, the body mostly obtains it from the dermal synthesis that occurs following Ultraviolet-B radiation. The main form of vitamin D found in the body is 25-hydroxyvitamin D, or 25(OH)D (calcidiol), which is produced by the enzymatic conversion of vitamin D3 (cholecalciferol) and D2 (ergocalciferol), which are derived from plants and animals, respectively, and the body, respectively. Serum 25(OH)D concentration, which should typically range from 30 to 100 ng/mL, is used to diagnose vitamin D deficiency. Values below 20 ng/mL are regarded as inadequate. It has also been shown that serum vitamin D levels and the prevention of cancer are closely related. The

serum level of 25(OH)D and several malignancies, including ovarian, breast, colon, and prostate tumours, are inversely correlated. Levels lower than 25 nM (10 ng/mL) were associated with higher colorectal cancer risk. Low levels (below 10 nM-4 ng/mL) are also connected with a high risk of cardiovascular disease and hypertension.

Different functions of vitamins maintain the body functioning effectively. While some vitamins support healthy neurons and infection resistance, others may aid in correct blood clotting or food energy absorption (3).

A fat-soluble vitamin known as vitamin D (also known as "calciferol") can be found as a dietary supplement and is added to some meals. Additionally, it is created internally when ultraviolet (UV) rays from sunshine strike the skin and start the production of vitamin D. (4)

The body needs to go through two hydroxylation processes in order to activate the biological inactive form of vitamin D that is received through sunlight, meals, and supplements. Vitamin D undergoes its first hydroxylation in the liver, resulting in 25-hydroxyvitamin D [25(OH)D], sometimes referred to as "calcidiol." The physiologically active 1,25-dihydroxyvitamin D [1,25(OH)2D], also known as "calcitriol," is created by the second hydroxylation, which largely takes place in the kidney.

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To permit regular bone mineralization and to avoid hypocalcemic tetany, which causes uncontrollable muscular contractions that result in cramps and spasms, vitamin D improves calcium absorption in the stomach and maintains adequate serum calcium and phosphate concentrations. Additionally, osteoblasts and osteoclasts use it to develop and remodel bone. Without enough vitamin D, bones may become fragile, deformed, or thin. Inadequate vitamin

D prevents osteomalacia in adults and rickets in youngsters. Vitamin D, together with calcium, aids in preventing osteoporosis in older people.

Other functions of vitamin D in the body include reducing inflammation and regulating cell growth, immune system activity, neuromuscular function, and glucose metabolism. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D. Many tissues have vitamin D receptors, and some convert 25(OH)D to 1,25(OH)2D.

The two primary forms of vitamin D found in foods and dietary supplements are D2 (ergocalciferol) and D3 (cholecalciferol), which only differ chemically in their side-chain configurations. The small intestine efficiently absorbs both types. Simple passive diffusion and a mechanism involving intestinal membrane carrier proteins both contribute to absorption. Although some vitamin D is absorbed even without dietary fat, absorption is enhanced when fat is present in the gut at the same time. Ageing and obesity have little impact on how well the gut absorbs vitamin D.

The key biomarker of vitamin D status at the moment is serum levels of 25(OH)D. It represents both naturally occurring vitamin D and vitamin D from dietary sources and supplementation. 15 days is a reasonably long half-life for 25(OH)D in serum. Serum concentrations of 25(OH)D are reported in both nanomoles per liter (nmol/L) and nanograms per milliliter (ng/mL). One nmol/L is equal to 0.4 ng/mL, and 1 ng/mL is equal to 2.5 nmol/L. (5)

In calcium homeostasis and bone metabolism, vitamin D is essential. A reduction in intestinal calcium and phosphorus absorption causes hypocalcemia, which causes secondary hyperparathyroidism in those with chronic and/or severe vitamin D deficiency. The result of this secondary hyperparathyroidism is phosphaturia and an expedited bone demineralization process. Additionally, this may lead to adult osteomalacia and osteoporosis and juvenile rickets and osteomalacia. (6)

The lack of understanding that moderate sun exposure is the main source of vitamin D for most persons is the main contributor to vitamin D deficiency. Very few foods naturally contain vitamin D, and those that are supplemented with it frequently fall short of meeting either an adult's or a child's needs. Rickets in children and osteopenia, osteoporosis, and fractures in adults are brought on by vitamin D insufficiency. Lack of vitamin D has been linked to an increased risk of viral disorders, hypertension, autoimmune diseases, and common

malignancies. To maximise vitamin D's positive effects on health, blood levels of the vitamin must be above 75 nmol/L, or 30 ng/mL. In the absence of adequate sun exposure, at least 800-1000 IU vitamin D3/d may be needed to achieve this in children and adults. Vitamin D2 may be equally effective for maintaining circulating concentrations of 25-hydroxyvitamin D when given in physiologic concentrations. (7).

1.2 NANOTECHNOLOGY

Numerous sectors, including medicine and physiology, promise to benefit significantly from breakthroughs in nanotechnology and nanoengineering. They can be broadly categorised as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organisation is on the nanometer scale, ranging from a few to several hundred nanometers, in at least one dimension. The size scale of a molecule itself is around one billionth of a metre, or three orders of magnitude, smaller than a micron. Since control over the underlying molecular structure enables control over the macroscopic chemical and physical properties at these scales, consideration of individual molecules and interacting groups of molecules in relation to the material's or device's bulk macroscopic properties becomes significant. Applications to physiology and medicine entail tools and materials made to communicate with the body at the subatomic level.

The spatial and temporal scales under consideration directly influence the possible impact of nanotechnology: Nanoscale engineering refers to the precise control of the arrangement of the constituent molecules and atoms that make up the bulk macroscopic substrate in materials and electronics. This implies that, as a result of the control over their molecular synthesis and assembly, nanoengineered substrates can be made to display very particular and controlled bulk chemical and physical properties. These materials and devices can be created to interact with cells and tissues at a molecular (i.e., subcellular) level with a high degree of functional specificity for applications in medicine and physiology, enabling a level of technological and biological system integration that was previously unachievable. It should be appreciated that nanotechnology is not in itself a single emerging scientific discipline but rather a meeting of traditional sciences such as chemistry, physics, materials science, and biology to bring together the required collective expertise needed to develop these novel technologies. (8).

The International Organisation for Standardisation (ISO), a global federation of national standards organisations, defines nanoparticles as nanoobjects with all exterior dimensions in the nanoscale and where the lengths of the longest and shortest axes of Nanoobjects do not significantly differ. Despite the fact that the nanoscale is essentially 1 to 100 nm in size, nanoparticles can be divided into three size categories: those bigger than 500 nm, those between 100 and 500 nm, and those between 1 and 100 nm. Nanoparticles may display size-related intense features depending on their size and size distribution. If they are small enough to contain their electrons, they exhibit surprising qualities and generate quantum effects, such as the red coloration of gold nanoparticles in solution.

The considerable changes in attributes associated to contact/surface area, such as catalytic, surface-enhanced Plasmon resonance, etc., are provided by the high surface-area-to-volume ratio of nanoparticles. Nanoparticles can have single properties like metallic, dielectric, semiconductor, magnetic, or multifunctional, which includes more than one feature from single-property nanoparticles, depending on the composition and structure. Their uses (or potential uses) can be found in numerous domains (and references therein). The benefits of nanoparticles in applications in the life sciences and the environment are attributable, among other things, to the fact that their size is equivalent with that of things like viruses (about 10-100 nm) or cells (about 1-10 m). This gives nanoparticles an ability to attach to biological entities without changing their functions, while the high surface-area-to-volume ratio of nanoparticles permits strong bonds with surfactant molecules.

Numerous processes, including chemical reduction, coprecipitation, seeding, microemulsion and inverse microemulsion, hydrothermal, and sonoelectrodeposition, are used to create nanoparticles. Additionally, coating or functionalizing materials like Silica An essential concern is the coating of magnetic nanoparticles employed to maintain the stability of MNPs over an extended period of time without agglomeration or precipitation. It is essential to safeguard MNPs from oxidation by oxygen and acid or base erosion. The typical approach involves shielding the particles with an impermeable barrier, preventing elements like oxygen from getting to their surface.

It should be noted that particle stabilisation and protection are frequently intertwined. MNPs can be shielded by having a silica coating applied to them. In addition to shielding the magnetic cores from outside agents that can interact unintentionally, a silica shell can stop the magnetic

core from coming into direct touch with other substances that are connected to the silica surface.Inorganic/inorganic, inorganic/organic, organic/organic, core/multishell, and moveable core/hollow shell nanoparticles are the several subtypes of core-shell structured nanoparticles. Top-down and bottom-up synthesis techniques can be used to create nanoparticles. The top-down approaches frequently employ externally controlled tools, such aslithography methods, laser beams, and mechanical techniques, to mill, cut, and shape materials into the required nanoscale structures. The bottom-up methods, like chemical synthesis, take advantage of the molecules' chemical characteristics to encourage self-assembly and the formation of nanoparticles. When opposed to top-down procedures, bottom-up techniques may create considerably smaller nanoparticles and are more economical. Core-shell structured nanoparticles are created using both techniques. However, the bottom-up strategy has proven to be more effective because ultimate control is required to achieve a homogeneous covering of the shell. It is also possible to combine the two approaches, for example, to create core particles using the top-down approach and then coat them using the bottom-up method to maintain a precise shell thickness. (9)

The creation of engineered objects at the atomic, molecular, and macromolecular levels in the nanoscale range is known as nanotechnology. Nanoparticles could be used for therapeutic and diagnostic purposes in the medical industry. For the diagnosis of infectious and cancerous diseases, nanotechnology devices are being developed, which can aid in the disease's early identification. Nanotechnology developments have also been helpful in the therapeutic fields of drug discovery, drug delivery, and gene/protein delivery. Nanoparticles can be created using a variety of techniques, allowing effects to be directed at specific locations. (10)

Comparison of different materials of Nano devices displaying the shapes, the sizes, the properties, and the use of different materials for diagnosis and/or therapeutics. (11)

Material	Size range	Shape	Properties	Applicability
Fullerene Carbon nanotubes	Nm	Nanotubes	carbon nanomaterials in molecular electronics	Nanowire and biosensor for diagnosis
Polylactic acid Polyethyleinemine Poly(cyano)acrylates Block copolymers Polycaprolactone	1–1000nm	Spherical	Biodegradable Biocompatible Smart material (external stimuli degradation pH, temperature…)	Drug/gene delivery
Gold nanoparticles	3–100 nm	Spherical	electronic, optical, and thermal properties	Diagnostics and detection of biological molecules at low concentration
Magnetic nanoparticles	3–100 nm	Spherical	Magnetic properties	Magnetic immunoassays, drug delivery, cell separation, purification, and tissue repair.
Quantum dots	1–10 nm	Spherical	Cd/Zn-selenides	In vitro diagnostic Imaging
Dendrimers	10–200 nm	Complex, branched polymer		Drug delivery systems

Table No. 1 Use of different Nano materials for diagnosis and therapeutics.

An analytical tool called a biosensor is used to find biological products. Nanotechnology is becoming more and more significant in the creation of biosensors. Nanobiosensor technology, which is based on nanotechnology, is revolutionising the healthcare sector. Forensic medicine and homeland security employ nanobiosensor technology to analyse metabolites, monitor diabetes, and other conditions. The introduction of numerous new signal transduction technologies has been made possible by the use of nanomaterials in the creation of biosensors, which has increased their sensitivity and performance. Sensors that interact with extremely small molecules that need to be studied have developed as a result of the development of equipment and techniques used to create, analyse, and picture nanoscale objects. Varies mechanical devices, optical resonators, functionalized nanoparticles, nanowires, nanotubes, and nanofibers-based Nano biosensor architectures have been used. A new interdisciplinary frontier between biological sensing and material science is the use of nanomaterials in biosensors, including gold nanoparticles, carbon nanotubes, magnetic nanoparticles, and quantum dots. It can be said that the development of ultrasensitive devices as a result of the advent of nanotechnology is probably one of the most promising ways to address some of the issues relating to the growing demand for highly sensitive, quick, and affordable analytical methods in the medical diagnostics, food and beverage industry, environmental protection, etc. (11).

1.3 BIOSENSAR

As per IUPAC, biosensor is defined as “A self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transducer element.” Biosensor is a device that combines a biological recognition element with a physical or chemical transducer detects a biological product. It is a probe that integrates a biological component with an electronic component to yield a measurable signal (14).

Biosensors consist of three basic components

(1) A bioreceptor (e.g., enzymes, antibody, microorganism, or cells);

(2) A transducer of the physicochemical signal, and

(3) A signal processor to interpret the information that has been converted. (12)

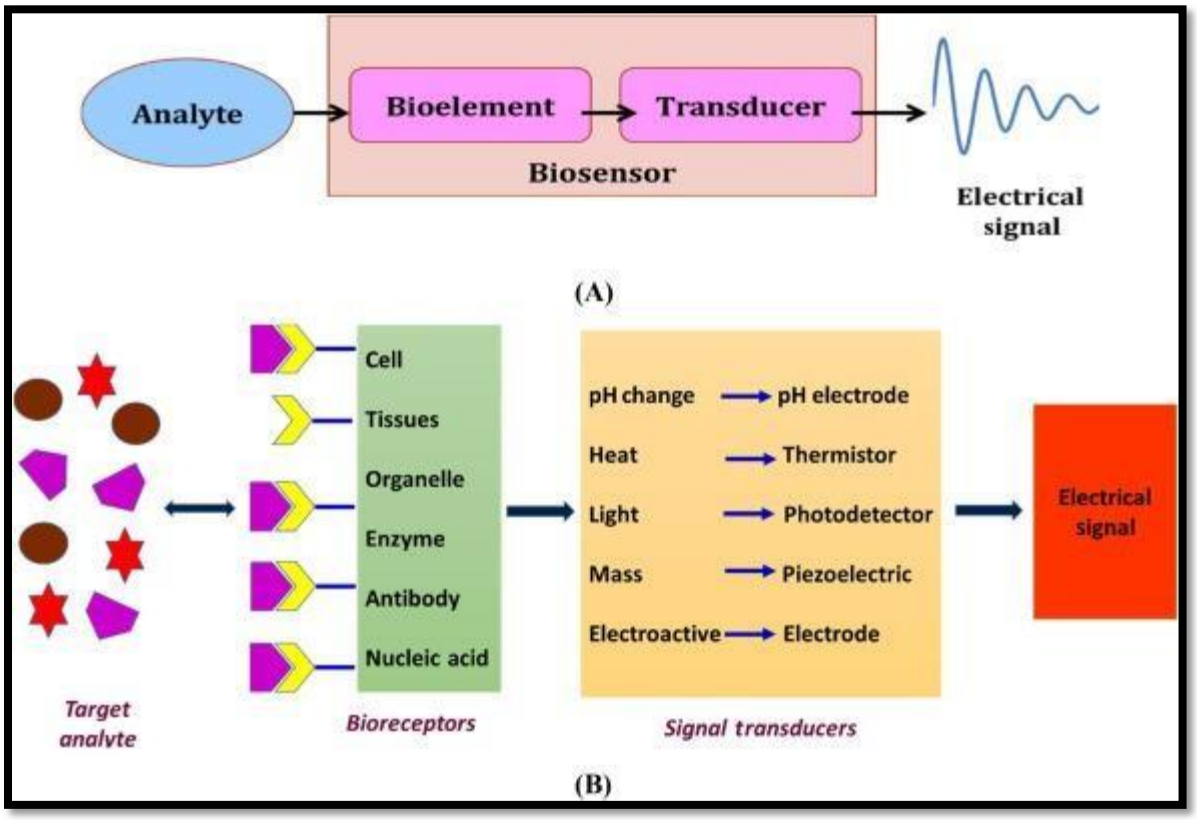

Fig.1 Components of Biosensor (13)

Basic characters of a Biosensor

1) Linearity: - Linearity of the sensor must be high for the detection of high substrate concentration

2) Sensitivity: - The value of the electrode response per substrate concentration

3) Selectivity: - Interference of chemicals must be minimised for obtaining the correct result

4) Response time: - The necessary time for having 95% of the response

Nanotechnology is not a single technique or study, but rather a broad term that covers a number of interdisciplinary fields including nanomaterials, devices, fabrication, electronics, communication, and energy. It is the capacity to gauge and regulate material at the nanoscale. The creation and modification of materials to Nano size (10^9m) is the subject of nanotechnology. Incorporating elements of material science, molecular engineering, chemistry, and biotechnology, nanomaterials-based biosensors have the potential to significantly increase the sensitivity and specificity of biomolecule detection, hold the ability to detect or manipulate

atoms and molecules, and have many applications, including biomolecular recognition, pathogen diagnosis, and environmental monitoring.

The nanotechnology products can be classified into three categories based on the number of dimensions:

(1) Thin films, such as coatings of implants for biocompatible purposes, anticoagulant coatings of stents, and coatings of pills and other therapeutic agents, have only one dimension

(2) Nanomaterials (NMs), such as carbon nanotubes (CNTs), silicon nanowires, nanorods, and fibres, have two dimensions.

(3) Nanomaterials (NMs), such as quantum dots, gold, magnetic and polymeric nanoparticles, and liposomes, have all the three dimensions.

The interest in incorporating various nanomaterials into transducers or receptor operation parts to strengthen biosensor technology's multi-detection capability and sensitivity is always growing, according to studies. Nanoparticles, nanotubes, quantum dots, and other biological nanomaterials are examples of these nanomaterials. These nanoparticles may support the transducer, the bio-recognition component, or both. In order to enable the quick analysis of numerous compounds in vivo, nanosensors, nanoprobes, and other nano devices have revolutionised the domains of chemical and biological analysis. A wide range of nanoparticles with various characteristics, including small size, fast speeds, shorter distances for electrons to traverse, reduced power, and lower voltages, have emerged in recent years. Nanomaterials like metal nanoparticles, oxide nanoparticles, magnetic nanomaterials, carbon materials, Quantum Dots, and metallophthalocyanines are now used as a result of significant advancements in the field of nanotechnology to enhance the electrochemical signals of biocatalytic events that take place at the electrode/electrolyte interface. For use in biosensors, functional nanoparticles that are attached to biological molecules (such as peptides, proteins, and nucleic acids) have been produced. The top-to-bottom method entails the use of physical (anisotropic) or chemical (isotropic) processes to micro- or nano-machine macroscopic materials down to the necessary nanoscale scale. This procedure combines lithography, laser ablation, ion milling, and chemical etching processes.

On the other hand, the material is "built" in the bottom-up method by first forming a critical mass and then accumulating material afterward. Molecular beam epitaxy, physical or chemical

vapour deposition and evaporation, and the (bio)chemical processes for the synthesis of (supra) molecular complexes, self-assembled monolayers, and protein-polymer nanocomposites are the techniques for bottom-up nanofabrication that are most frequently utilised. In the past ten years, a number of intriguing NMs have been applied to diagnostics and biosensors, including carbon nanotubes (CNTs), graphene, quantum dots (QDs), nanoparticles (NPs), and nanocomposites.

Materials with Nanostructures for Biosensing Devices Due to quantum-size effects and their enormous surface areas, which offer distinct and varied features from bulk materials, nanostructured materials are well known as intriguing tools with specific physical and chemical properties. Exploring these various traits offers the chance to enhance biosensors' capabilities and boost detection strength through size and morphology management. When metallic nanostructures are utilised as parts to modify electrodes, interesting ways have documented a significant boost in electronic characteristics. Utilising nanostructured materials with particular shapes, such as 0D (quantum dots, nanoparticles), 1D (nanowires or carbon nanotubes), or 2D (metallic platelets or graphene sheets) orientation that reflects in their final attributes is one of them.

CNTs, or carbon nanotubes CNTs have been one of the most widely employed NMs in biosensors, diagnostics, tissue cell tracking and labelling, and medication and biomolecule delivery over the past ten years. They are single-, double-, or multi-walled CNTs, which are hollow cylindrical tubes made of one, two, or more concentric graphite layers and capped by fullerene spheres. Their distinctive architectures, superior electrical and mechanical qualities, high thermal conductivity, high chemical stability, impressive electrocatalytic activity, little surface fouling, low overvoltage, and high aspect ratio (surface to volume) are all among their many outstanding qualities. For the highly sensitive detection of analytes in healthcare, industry, environmental monitoring, and food quality analysis, CNTs-based biosensors and diagnostics have been used.

Nanoparticles (NPs) Due to their distinct optical and other features, NPs have also been widely used in numerous bioanalytical applications, including for the creation of biosensors, diagnostics, imaging, medication administration, and therapy. The attachment of molecules to their surface causes them to change colour. For a variety of bioanalytical applications, the ability of nanoparticles to change their properties by altering their size or shape has been used.

The most popular NPs are GNPs, which have an inert, nontoxic, and biocompatible core. For the early stage detection and photothermal therapy of cancer and other disorders, GNPs' strong plasmon absorption and scattering capabilities are very helpful. They have been applied to the creation of biosensors, diagnostics, and immunoassays for a variety of analytes.

Application of Nanotechnology Based Biosensor Technology

From the estimation and diagnosis in the health related in vivo aspects, biosensors can also be used for environment monitoring of pollutants, toxicants and physical aspects like humidity, heavy metal toxicity, and even presence of their carcinogens.

Biomedical and Diagnostic Application:

Biosensors have been used for biological detection of serum antigen and carcinogens, and causative organism of so many metabolic disorders since a long time.

The routine application in diagnosis is best described by the use of biosensors in the detection of disorders like diabetes, cancer, allergic responses, and so many other disorders on the basis of serum analysis. Biochips and microarray based technique has enabled the testing of many diseases in no time. With controlled synthesis, even magnetic nanoparticles have been synthesised and used for isolation and heavy metal resembling in properties with iron from the blood serum of living organisms.

Advantages of Nanotechnology Based Biosensors

- It is a detection of target molecules, a key factor in early detection of diseases such as breast cancer an AIDs
- Rapid and high throughput detection
- Detection processes are simple, user friendly, fast and cost effective
- Reduced material requirement to fabricate and easier recycling
- Novel properties and new capabilities
- Repetitive, portable and stability
- Nanotechnology are also being used in the detection of pathogen in environment.
- It is being used in toxicity analysis. (14)

1.4 APTAMERES

Aptamers have gained interest in the biosensing community as signal recognition components because to their strong binding affinities for a variety of specialised targets, including proteins, cells, tiny compounds, and even metal ions, for which it is challenging to find antibodies. Aptamers are single oligonucleotides that are produced via systematic evolution of ligand exponential enrichment (SELEX), an in vitro selection method. Aaptasensors are aptamers that have been functionalized and designed to provide many signalling modes, including colorimetric, fluorometric, and electrochemical, in addition to their high binding affinity. Recent developments in aptasensors have made them potent biosensor probes that may be employed in a variety of settings, such as drug monitoring, environmental monitoring, and clinical diagnostics.

Aptamers consist of 3D-folded structures of single-stranded oligonucleotides with lengths of usually 20–60 bases of nucleotides selected in vitro via the systematic evolution of ligand exponential enrichment (SELEX) process. The SELEX process is applicable for single targets, complex target structures, or even mixtures without proper knowledge of their composition. SELEX can be used to select aptamers with high affinities and specificities for their targets and low dissociation-constant values, across the low nanomolar to picomolar range. Aptamers can be selected through the in vitro process independent of animals or cell lines. The selection of aptamers for toxic target molecules or molecules with no or low immunogenicity is possible. Different modifications can be introduced in the basic SELEX process for the selection of the desired aptamer specifications for a specific application. The aptamers can be functional for native conformations of target molecules on live cells, so cell surface transmembrane proteins can be considered as targets (15).

Due to their benefits over antibodies, including excellent stability, low dimension, and strong affinity for tiny compounds, they have been exploited as novel recognition elements in sensors swiftly. Additionally, they are simple to alter, straightforward to synthesise in vitro, and versatile to design in the sequences. These inherent advantages of oligonucleotides can enhance detection through effective, exceptional binding constants (in the micromolar to picomolar range), in addition to assisting with target diversification. They can also assist analysts in putting together useful and smart sensing platforms. All of these factors led to the rational design of a wide number of aptamer sensors, which utilised a variety of approaches or

combined them to provide a detectable signal that was suitable (optical, electrochemical, piezoelectric, etc.). In the last decades, nanomaterials have found wide applications in sensor technology because they can greatly facilitate the miniaturization of instrumentation and improve the analytical performance; in addition, they have unique electrochemical properties. In fact, nanostructures increase the electron transfer between the redox center and the electrode surface. Moreover, the number of immobilized bioreceptors increases because of their high surface/volume ratio (16).

Aptamers for Biomedical Diagnostics: Due to the high affinity to their targets, aptamers can be developed as diagnostic tools. In addition, aptamers possess the advantages of easy conjugation and labeling features, which enable them to be easily combined with other novel techniques, such as endogenous nucleic acid analysis, microfluidic cell separation, flow cytometry or nanoparticle-based sensing to maximize their diagnostic functions. The first aptamer used as a diagnostic tool was developed in 1999 by Bruno et al. They used the aptamer selected against *Bacillus anthracic* spores to detect anthrax spores. To date, aptamers have been widely applied for the diagnosis of ophthalmology, cardiovascular diseases or cancer diseases (17).

2. REVIEW OF LITERATURE

1. **Woochang Kim et. al. (2021)** were developed a bio-inspired silver nanovilli-based sandwich-type surface enhanced Raman scattering aptasensor for the ultrasensitive and selective detection of 25- hydroxy vitamin D3. The sandwich-type aptasensor was designed using an aptamer and 4-Phenyl-1,2,4-triazoline-3,5-dione-methylene blue complex. The nanovilli-based aptasensor could sensitively detect various concentrations of 25-hydroxy vitamin D3, ranging from those found in deficient to excess conditions.
2. **Ritika Gupta et.al. (2021)** developed using aptamer and graphene oxide (GO). Aptamer is an oligonucleotide, sensitive towards its target, whereas, GO with 2D Nano sheets provides excellent quenching surface. Aptamer labeled with fuorescein (5', 6-FAM) is adsorbed by π–π interaction on the GO sheets leading to quenching of the fluorescence dueto Förster resonance energy transfer (FRET). However, in the presence of 25(OH) D3, a major portion of aptamer fluorescence remains unaltered, due to its association with 25(OH)D. The limit of detection of 25(OH)D3 by this method was found to be 0.15 µg/mL.

3. **Tamal Sarkar et.al. (2017)** had reported detection vitamin D2, a biosensing platform based on chitosan (CH) and modified with carbon dots (CDs) was created. Microwave pyrolysis was used to create the carbon dots, which were then examined using transmissionelectron microscopy, Raman spectroscopy, Fourier transform infrared spectroscopy, and UV/VIS spectroscopy. BSA/Ab-VD2/CD-CH/ITO bio electrode was created by further modifying the CD-CH/ITO surface by immobilising vitamin D2 antibody (Ab-VD2) and bovine serum albumin (BSA). By using differential pulse voltammetry, the electrochemicalresponse of the bioelectrode to the vitamin D2 antigen (Ag-VD2) was measured. The biosensing electrode displayed linearity in the Ag-VD2 concentration range of 10–50 ng mL1.
4. **Mohammad Ali et. al. (2023)** had developed an electrochemical sensor for rapid detection of 25 OH D3 based on the modification of glassy carbon electrode by nanocomposite of $CuCo_2O_4$/nitrogen-doped carbon nanotubes and phosphorus-doped graphene oxide. Then 25-hydroxyvitamin D_3-imprinted polypyrrole was coated on the electrode surface through electro polymerization. They have detectected 25 OH D3 in serum samples in the range of 0.002–10 μM, with a detection limit of 0.38 nM.
5. **Francesca Polli et. al. (2023)** have reported a new sensitive label-free electrochemical immunosensor to detect Vitamin D_3 (25-OHD_3) in untreated serum samples, For this a graphite screen printed electrode (SPE) was modified using cysteamine (CYM) functionalized core-shell magnetic nanoparticles (Au@MNPs) then, the 25- OHD_3 antibody (AbD) was immobilized via glutar aldehyde crosslinking. Analysis was monitiored by using differential pulse voltammetry (DPV). LOD of 2.4 ng mL^{-1} and a linear range between 7.4 and 70 ng mL^{-1}.
6. **Amandeep Kaur et. al. experiment (2023)** based on advanced sensing platform composed of meso-microporous silica-zeolitic imidazolate framework-8 (MSS-Z8) with highly enhanced surface area ($S_{BET, MSS-Z8}$ (643.4 m^2g^{-1}) > SBET, MSS (49.95 m^2g^{-1})), embedded with gold particles. Analysis was performed by using differential pulse voltammetry (DPV).
7. **Thiago S. Martins (2022)** have reported a flexible and label-free immunosensor chips made with tree-like gold dendrites (AuDdrites) electrochemically formed by selective desorption of l-cysteine (*L*-cys) on (111) gold planes. The 25-hydroxyvitamin D3

(25(OH)D3) metabolite was detected within 15 min with a limit of detection (LOD) of 0.03 ng mL^{-1}.

8. **Bang Hyun Lee et.al. (2017)** from their study they have reported Using the immobilization-free, graphene oxide-based systemic evolution of ligands by exponential enrichment method, they successfully developed aptamers that can bind to 25-hydroxyvitamin D3 with high specificity and affinity in order to detect 25-hydroxyvitamin D3 deficiency. Based on the specificity and affinity of the aptamers confirmed by both the gold nanoparticles (AuNPs)-based colorimetric assay and the isothermal titration calorimetry (ITC) method, the 9 sequences, including the VDBA14 aptamer, were obtained out of 16 aptamer candidates.

9. 2.1 REPORTED RESEARCH PATENTS ON BIOSENSORS

1. **Dennis S. Fernandez, patented** (Pub. No. US 2005/OO43894 A1) INTEGRATED BIOSENSOR AND SIMULATION SYSTEM FOR DAGNOSIS AND THERAPY integrated biosensor-simulation system combines one or more sensors that can identify different situations in a biological target or host with software that uses a system biology model and sensor data adaptively to give therapy, diagnosis, or other automated feedback.

2. **G. Tayhas R. Palmore, patented** (Pub. No. US 2012/0183988 A1) INTEIN-BASED FLUORESCENT BO-CIRCUIT FOR VITAMIND DETECTION an intein -based vitamin D fluorescent biosensor that accurately and rapidly measures 1α 25-hydroxyvitamin D3. Measurement at picomolar quantities is noted.

3. **Menno Willem Jose Prins patented** (Pub. No. US 9,128,084 B2) FAST BIOSENSOR WITH REAGENT LAYER A detection system and a sensor chip for detecting target molecules, and thus corresponding analytes in a sample and detection system includes a sensor chip.

4. **Michael R. Ladisch, patented** (Pub. No.US 2003/0036054A1) BIOSENSOR AND RELATED METHOD A Micro Scale biosensor for use in the detection of target biological Substances including molecules and cells is a microfluidic System with integrated electronics, inlet-outlet ports and interface Schemes, high Sensitivity detection of pathogen Specificity, and processing of biological materials at Semiconductor interfaces.

2.3 DETECTION TECHNIQUES AVAILABLE FOR VITAMIN D

Several methods have been routinely used in the quantification of vitamin D, including competitive protein-binding (CPB) assays, radioimmunoassay (RIA), chemiluminescence immunoassays (CLIA), liquid chromatography (LC) with UV detection, and liquid chromatography-mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS). In clinical practice, CPB, RIA and CLIA remain the most widely applied assays.

3. AIM AND OBJECTIVES

3.1 AIM: Detection of Vitamin D by Nanotechnology.

3.2 OBJECTIVES:

1. To synthesis / procure nanoparticle.
2. To characterize nanostructure by using modern instrumental tool.
3. Design and development of biosensor/tool/software for detection of vitamin D.
4. Toxicity detection.
5. Validation of biosensor/tool/software.

3.3 PROPOSED HYPOTHESIS

1. Vitamin D plays major role in immunity, inflammation, metabolism of carbohydrates, Fat and protein etc. deficiency of Vitamin D may lead to develop various diseases, in order to maintain proper health minimum requirements of daily vitamin D is required.

3. India having 1.4 billon population, among which 40-90% peoples are suffering from vitamin D deficiency, although various techniques are available for detection of vitamin D like Atomic absorption spectroscopy, UV-Visible spectroscopy, Colorimetric analysis, Atomic Emission Spectroscopy photoimmuloscence, chemimmuloscence etc. these techniques are complex, time consuming and expensive therefore there is need to developed new method such as biosensor for detection of vitamins. Biosensors are rapid, simple & inexpensive device with high sensitivity and bio recognition element for analyte specificity. so from a literature review, to ease the detection of vitamin D levels we have decided to develop biosensor to detect Vitamin D by nanotechnology.

4. METHODOLOGY

1. Extensive literature survey
2. Synthesis / Procurement of nanoparticle.
3. Characterization of nanostructure by using modern instrumental tool.
4. Development of recognition element/ transducer.
5. Toxicity detection.
6. Validation biosensor/tool/software.

5. IMPORTANCE OF STUDY:

- A varieties of techniques have been applied to examine vitamin D such as atomic absorption spectrometry, antibody-based detection, electrochemistry, UV-Vis spectroscopy, ion chromatography, colorimetric analysis, atomic emission spectrometry, inductively coupled plasma, and fluorescence spectroscopy.
- However, these techniques are complex, expensive and time-consuming.
- Therefore, there is a need to develop new methods, such as biosensors for detection of vitamin D.
- Biosensors are rapid, simple, and inexpensive devices with high sensitivity and bio recognition elements for analyte specificity.

5.1 INTERNATIONAL STATUS

- One of the most prevalent medical conditions worldwide is vitamin D insufficiency. Poor bone growth and health are side effects of vitamin D insufficiency, as is an increased risk of many common and serious illnesses like type 1 diabetes, some types of cancer, and other autoimmune and cardiovascular problems.
- In the US, Canada, New Zealand, Australia, Europe, and Asia, it has been estimated that between 30-50 % of children and adults suffer from vitamin D insufficiency.
- While over 1 billion people around the world are estimated to be vitamin D deficient, over 50% of the population are projected to have insufficient levels of vitamin D.
- Several epidemiological research suggests that about 24% of people in the US are vitamin D inadequate. Other regions of the globe may have higher rates of deficiency.

- Rickets is more common among children and causes softening of bones, skeletal fractures, and deformity. Depression, bone pain, and weakness are other associated conditions with the disease.
- Such factors propel the awareness of Vitamin D, thus boosting the Vitamin D Market share globally. (18)

5.2 NATIONAL STATUS

- Low levels of vitamin D in the body can lead to loos of bone density, which increases risk of Osteoporosis and fractures.
- Severe deficiency can lead to rickets in kids, which is a condition that affects bone development in children.
- Vitamin D deficiency with other health conditions including diabetes, high blood pressure, cancer, and autoimmune conditions such as multiple Sclerosis.
- Nearly 76% of the Indian population suffers from vitamin D deficiency, according to data from tests more than 2.2 lakh people conducted across 27 cities in India by Tata 1 mg labs recently (19).

6. RESEARCH WORK PLAN:

Sr. No.	Work	Period
1	Literature survey	6 months
2	Synthesis/ procurement of Nanoparticle and characterization of Nanostructure.	6 months
3	Development of recognition element/ transducer.	6 months
4	Toxicity detection	6 months
5	Validation biosensor/tool/software.	6 months
6	Other suggested work and compilation of data	6 months
	Total duration of work	36 months

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